

AD_____

Award Number: W81XWH-12-1-0245

TITLE: Evaluation of Multimodal Imaging Biomarkers of Prostate Cancer

PRINCIPAL INVESTIGATOR: Christopher Chad Quarles

CONTRACTING ORGANIZATION: Vanderbilt University
Nashville, TN 37203

REPORT DATE: September 2013

TYPE OF REPORT: Annual report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE September 2013		2. REPORT TYPE Annual		3. DATES COVERED 1 September 2012-31 August 2013	
4. TITLE AND SUBTITLE Evaluation of Multimodal Imaging Biomarkers of Prostate Cancer				5a. CONTRACT NUMBER W81XWH-12-1-0245	
				5b. GRANT NUMBER W81XWH-12-1-0245	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Christopher Chad Quarles E-Mail:				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Vanderbilt University Medical Center Nashville, TN 37203				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The goals of the proposed studies are to: i) use imaging methods to non-invasively assess the temporal relationship between castration resistant prostate cancer (CRPC) growth, androgen receptor (AR) levels, angiogenesis, hypoxia, and translocator protein (TSPO) levels and ii) use imaging to temporally direct pathological examination of tissue in order to enhance the elucidation of mechanistic aspects of CRPC progression, specifically the involvement of HIF-1alpha and NF-kappaB, two pathways that increase AR activity during progression to CRPC. As described in the statement of work, the first year of this award focused on implementation and optimization of the imaging methods in the Pten / p53 double null mutant mouse model. Towards that end, we have successfully optimized anatomic MRI and dynamic contrast enhanced (DCE) MRI methods, in orthotopic tumors within the Pten/p53 mouse model, to assess tumor volume, track growth and tumor angiogenesis. In regards to PET imaging, we have completed the synthesis, purification and stability verification of the [18F]-fluoro-5alpha-dihydrotestosterone (FDHT) radiotracer, which will be used to assess AR levels in these mice. We have also acquired dynamic FDHT PET data in control mice and those bearing prostate cancers. At this stage in the project we have finalized the MRI and PET acquisition protocols that will be utilized in the second year of the award.					
15. SUBJECT TERMS castration resistant prostate cancer, MRI, PET, FDHT, image optimization					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	10	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	2
Key Research Accomplishments.....	7
Reportable Outcomes.....	8
Conclusion.....	9

Introduction

In its advanced stages, prostate cancer (PCa) becomes clinically difficult to restrain due to failure of therapy and the development of castration resistant prostate cancer (CRPC). Thus, there is a compelling need to investigate the mechanisms leading to CRPC in order to develop more effective treatment strategies. The most common approach to biologically assess disease progression in mouse models of PCa is through pathological examination, which requires the sacrifice of mice at multiple arbitrary time points and, consequently, is unsuitable for the temporal characterization of physiological, cellular and molecular events leading to CRPC growth in a given animal. In recent years, however, there have been dramatic increases in the range and quality of information available from non-invasive imaging methods so that many potentially valuable imaging metrics are now available to quantitatively measure tumor growth, assess tumor status, and predict treatment response. To this end, our study aims to evaluate emerging, clinically-viable imaging metrics in an appropriate PCa animal model to serially assess tumor progression and establish which method (or combination of methods) is most accurate at predicting castration induced tumor regression and the subsequent recurrence of the castration resistant tumors. In particular, we proposed to non-invasively assess the temporal relationship between CRPC growth, androgen receptor (AR) levels, angiogenesis, hypoxia, cellular proliferation and apoptosis using Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET). Such studies could provide the scientific basis for the acceleration of these emerging imaging methods into clinical care and could have a direct impact on prostate cancer detection, staging and treatment monitoring. Additionally, we proposed to use multiparametric imaging to temporally direct pathological examination of tissue in order to elucidate mechanistic aspects of CRPC progression, specifically the involvement of hypoxia (HIF-1 α) and NF- κ B, two essential pathways that increase AR activity during progression to CRPC. The proposed studies are being carried out in the genetically engineered *Pten/p53* conditional mouse model (*Pten*^{pc}^{-/-}; *Trp53*^{pc}^{-/-} double-null mutants). Our preliminary studies revealed that prostate tumors in these mutant mice are initially sensitive to castration, as evidenced by tumor regression, but this is followed by tumor recurrence that is ultimately lethal. The regression response to castration and subsequent CRPC growth in these mice clinically recapitulate the disease progression observed in human prostate cancer undergoing androgen-ablation therapy (AAT). Therefore, this authentic mouse model provides a valuable and unique tool to study CRPC progression and dysregulated pathways that could be used as targets of novel therapeutic strategies. Our hypothesis is that multiparametric imaging of vascular, cellular and molecular events will identify stages that predict CRPC progression.

Body

The overall goal of the project in Year 1 was to implement and optimize the imaging methods that will be used to track CRPC growth and treatment response in Years 2 and 3. In the Statement of Work, Tasks 1 – 4 describe the studies proposed in Year 1. These tasks along with the corresponding progress statements are described below.

Task 1: Synthesize 16 β -18F-Fluoro-5 α -Dihydrotestosterone (Months 1 - 8)

1a: Purchase materials for synthesis, synthesize precursor and reference standard compounds (Months 1 – 4)

1b: Develop radiolabeling conditions for synthesis and purification of [18F] FDHT (Months 5 - 7)

1c: Verify stability of radiotracer *in vitro* and *in vivo* (Month 8)

Progress: In the original proposal of this project we indicated that the Vanderbilt Molecular Imaging and Radiochemistry core would synthesize the FDHT agent needed for these studies using previously reported methods. Based on their recommendation, rather than pursuing a new precursor synthesis we elected to purchase the FDHT precursor from the **Radiochemistry & Molecular Imaging Probe Core** at Memorial Sloan Kettering. This enabled us to focus our efforts primarily on the Tasks 1b and 1c and forego any unforeseen issues with the tracer synthesis.

Conditions for radiosynthesis of 18 β -[18F]fluorodihydrotestosterone ([18F]FDHT) were developed using a combination of an automated reaction module (GE TRACERlab FX_{F-N}) and manual manipulations. Preparation (**Scheme 1**) began with standard nucleophilic radiofluorination (step 1) of protected precursor 16 α -[[[(trifluoromethyl)sulfonyl]oxy]-3,3-(ethylenedioxy)androstano]-17-one (Memorial Sloan-Kettering Cancer Center) within the reaction module. After completion of radiofluorination, the material was then transferred out of the module and reduced *via* lithium aluminum hydride (LiAlH₄) at -78 °C (step 2). This was followed by reaction quenching and then deprotection using standard acid hydrolysis at 55 °C (step 3) to reveal the final product. The final product was then purified by semi-preparative HPLC (Hitachi High Technologies) and formulated in a solution containing no greater than 10% ethanol in saline.

Quality control was performed by analytical HPLC (Hitachi High Technologies) with inline UV and radiation detectors enabling comparison of the radioactive product to the reference standard ([19F]FDHT) (Figure 1). Results showed a perfect overlay with a typical radiochemical purity greater than 98%.

Scheme 1: Synthetic route towards [18F]FDHT.

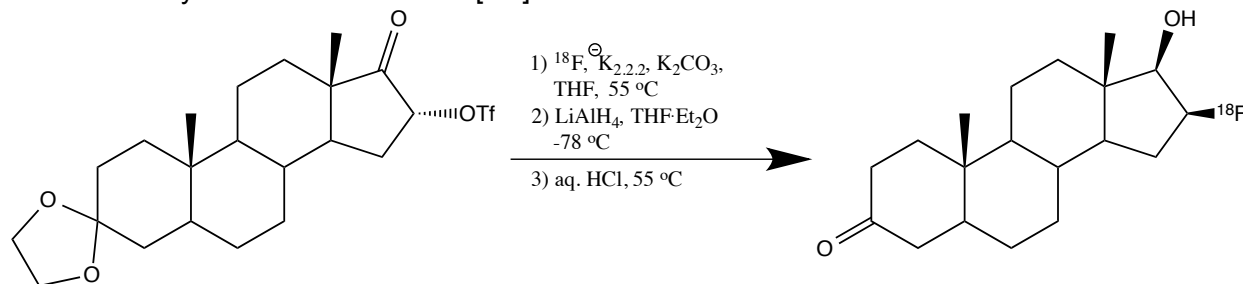
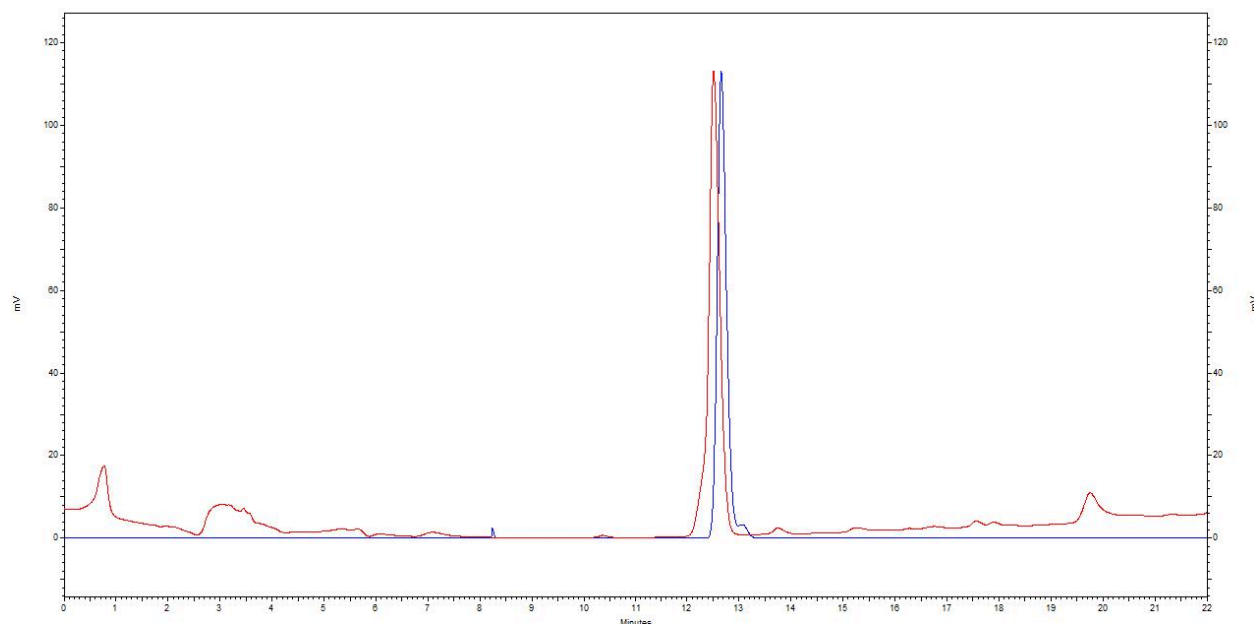


Figure 1: Overlay of the radioactive HPLC trace (Blue) and reference standard UV trace (Red). Retention time difference is due to the time delay between the UV detector and the radiation detector.



Task 2: Initiate mouse breeding to create Pten/p53 conditional mice (Months 1 - 12)

2a: Expand mouse colonies at Meharry to obtain breeding adult mutant mice (Months 1 - 3)

2b: Initiate breeding studies : PtenLoxP/LoxP; Trp53LoxP/LoxP female mice will be crossed with PtenLoxP/+; Trp53LoxP/LoxP; PB-Cre4 male mice to obtain PtenLoxP/LoxP; Trp53LoxP/LoxP; PB- Cre4 male mice (referred as Pten/p53 conditional double null mice) (Months 3 – 6)

2c: Transfer the first set of Pten/p53 conditional double null mice (n = 5) to Vanderbilt (Month 6)

2d: Continue breeding to create additional Pten/p53 conditional double null mice (Months 6 – 12)

2e: Transfer the second cohort of Pten/p53 conditional double null mice (n = 10) to Vanderbilt (Month 12)

Progress: Dr. Zhenbang Chen, Co-investigator at Meharry, successfully expanded his mouse colonies of the Pten/p53 conditional mice and was able to provide the mice needed for studies in Year 1. As he routinely uses these mice for other studies this colony is maintained and readily available for our studies going into Year 2. To date, 6 mice have been transferred to Vanderbilt (those described in 2c) and within the month the next cohort (described in 2e) will be transferred.

Task 3: Required Vanderbilt quarantine for initial cohort of animals (Months 6 - 7)

Progress: Consistent with Vanderbilt Division of Animal Care's policy we quarantined all mice that were transferred from Meharry to Vanderbilt for a period of 6 to 8 weeks. At the end of

quarantine all the mice tested negative for any contaminants and were transferred to the general animal housing facilities at Vanderbilt.

Task 4: Optimize MRI / PET prostate cancer imaging protocols (Month 8 - 12)

4a: Acquire serial anatomical MRI and dynamic contrast enhanced (DCE)-MRI images in prostate tumor-bearing Pten/p53 conditional mice (n = 5) to optimize imaging protocols and characterize tumor growth. (Months 8 – 9)

4b: Acquire dynamic PET FDHT and CT images in prostate tumor-bearing Pten/p53 conditional mice (n = 5) to characterize kinetics of tracer uptake and retention in this model. (Month 9)

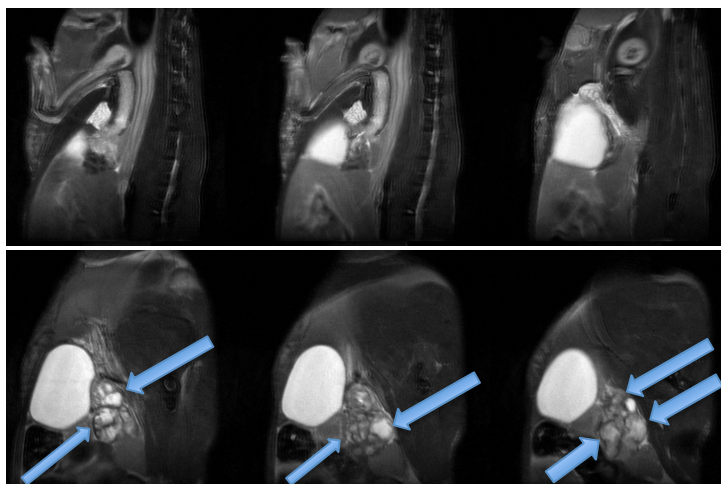
4c: Acquire dynamic PET FMISO and CT images in prostate tumor-bearing Pten/p53 conditional mice (n = 5) to characterize kinetics of tracer uptake and retention in this model. (Month 9)

4d: Acquire dynamic PET TSPO and CT images in prostate tumor-bearing Pten/p53 conditional mice (n = 5) to characterize kinetics of tracer uptake and retention in this model. (Month 9)

4e: Analyze MRI / PET data to finalize imaging acquisition protocols (Months 10 -12)

Progress: In the Pten/p53 conditional mice we were successfully able to use MRI to identify the prostate and developing tumors in three mice. In the other mice tumors did not develop. After animals were released from quarantine we were able to track the growth of multiple tumors over the course of two to three months. The primary goal of each of these scans was to optimize the acquisition protocols needed to: i) minimize motion artifacts, ii) reliably detect the prostate and any associated tumors, and iii) enhance contrast within tumors. Robust image quality and tumor detection was best achieved with a 2D T₂-weighted high-resolution fast spin echo scan with the following imaging parameters (Varian 7T MRI system, TR = 2s, TE = 40ms, echo train length = 8, echo spacing = 10ms, matrix = 256 x 256, FOV = 25.6 x 25.6 mm, 20 slices, 1 mm slice thickness, number of excitations = 20, scan duration ~ 21 min). We also have found that placing the animal on its back (rather than the usual stomach position) enabled us to avoid the use of respiratory gating in the scans, thereby reducing scan durations. It also reduced scan-to-scan variability in the location and appearance of the tumors. This feature also made the acquisition of the DCE-MRI data much more reliable as it removes any confounding issues that could occur due to variable breathing rates. **Figure 2** shows example images of a Pten/p53 mouse tumor. Across the small cohort of mice in this study, the number of tumors, their size, appearance and location was heterogeneous.

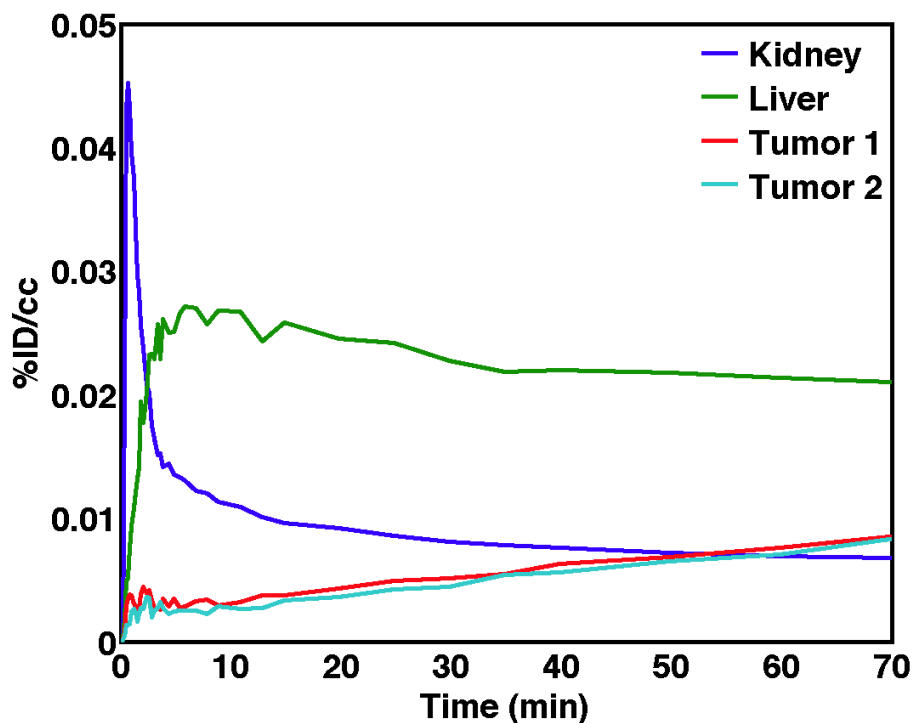
Figure 2: Example MRI images in a control (Top) and Pten/p53 mutant (Bottom) Mouse. Multiple tumors can be seen in the Pten mice as indicated by the blue arrows.



Given the length of time spent on optimizing MRI exams, our initial cohort of mice began to expire. We have ample prior experience with FMISO and TSPO in other animal tumor models, so we focused our remaining efforts on acquiring dynamic FDHT PET data in the remaining two mice. PET imaging was performed on a Concorde Microsystems microPET Focus 220. Approximately 120 uCi of FDHT was administered via an indwelling jugular catheter and dynamic scans were acquired for 2 hours. CT data was acquired on an ImTek MicroCAT II in approximately eight minutes using the following imaging parameters: voltage of 80 kvp with an anode current of 500 μ A, 360 projections in 1° steps, exposure time = 600 ms, acquisition matrix = 512³. **Figure 3** shows example FDHT kinetic curves in kidney, liver and tumors within a Pten/p53 mouse. As expected, the passage of the agent through the kidney exhibits rapid wash-in/out kinetics consistent with perfusion with little to no filtration. In contrast, the liver exhibits metabolism and retention of the agent consistent with this being the organ of excretion. The tumors show lower perfusion (low initial height) but a steady increase in uptake indicating binding and retention.

In the next cohort of animals transferred to Vanderbilt we will acquire FMISO and TSPO data in order to characterize the kinetics of tracer uptake in the Pten/p53 model. We will also perform a cold injection FDHT study following radiotracer injection to further verify specificity of the retained agent. These studies will not interfere with our currently planned efforts in Year 2.

Figure 3: Example FDHT kinetic curves in kidney, liver and two tumor regions of interest.



Personnel receiving pay from this research effort

C. Chad Quarles, Department of Radiology, Vanderbilt University (10% effort)

Dan Ayers, Department of Biostatistics, Vanderbilt University (5% effort)

Zhenbang Chen, Department of Biochemistry and Cancer, Meharry Medical College

Wenfu Lu, Department of Biochemistry and Cancer, Meharry Medical College

Key Research Accomplishments

- Optimization of high-resolution anatomic and contrast enhanced MRI methods to track tumor growth and angiogenesis in Pten/p53 mouse models
- Synthesis of the AR-imaging agent FDHT
- First FDHT PET images of androgen receptor levels in the Pten/p53 mouse model

Reportable Outcomes

Given that Year 1 was primarily focused on implementation and optimization no reportable outcomes are noted. We expect our first publication to come when we have acquired sufficient FDHT and MRI data in the Pten/p53 model (in year 2) since this will be the first non-invasive assessment of tumor AR levels before and after castration.

Conclusions

In Year 1 we have successfully completed nearly all of the elements on the approved Statement of Work. It is noteworthy, that we have optimized the imaged methods required to serially assess tumor growth, angiogenesis, and AR levels in the Pten/p53 model. Going into year 2 the combination of these methods with imaging metrics that interrogate tumor hypoxia and TSPO expression will enable a more comprehensive and serial characterization of castration resistant prostate cancer progression. If successful, these efforts will also provide the scientific basis for the acceleration of these emerging methods into clinical care and could have a direct impact on prostate cancer detection, staging and treatment monitoring.